Hallmarks of Atopic Skin Mimicked In Vitro by Means of a Skin Disease Model Based on FLG Knock-down

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Summary — Loss-of-function mutations in the filaggrin gene (FLG) are a strong predisposing factor for atopic dermatitis, although their relevance to the disease pathomechanism needs further elucidation. The generation of an in vitro model of atopic skin would not only permit further evaluation of the underlying pathogenetic mechanisms and the testing of new treatment options, but would also allow toxicological studies to be performed in a simple, rapid and inexpensive manner. In this study, we have knocked down FLG expression in human keratinocytes and created three-dimensional skin models, which we used to investigate the impact of FLG on epidermal maturation and on skin absorption and its response to irritation. Histopathological evaluation of the skin models showed impaired epidermal differentiation in the FLG knock-down model. In addition, skin irritation induced by an application of sodium dodecyl sulphate resulted in significantly higher lactate dehydrogenase leakage, and interleukin (IL)-6 and IL-8 levels, than in the control model. To assess the effect of filaggrin deficiency on skin absorption of topically applied agents, we quantified the percutaneous absorption of lipophilic and hydrophilic model drugs, finding clinical relevance only for lipophilic drugs. This study clearly demonstrates that important clinical characteristics of atopic skin can be mimicked by using in vitro skin models. The FLG knock-down construct is the first step toward an in vitro model that allows clinical and toxicological studies of atopic-like skin.

Key words: atopic dermatitis, filaggrin, in vitro skin disease model, siRNA, skin barrier function.

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Introduction

Atopic dermatitis (AD) is a major public health problem of the Western world. Currently, about 10–20% of children and 1–3% of adults are affected, but its prevalence is increasing. AD exhibits a multifactorial disease pattern, the pathophysiology of which is not yet fully understood (1, 2). To date, it is known that gene mutations, such as loss-of-function mutations in the filaggrin gene (FLG), the individual’s environment, and immunological factors, such as increased levels of T helper 2 (Th2) cytokines initiated by, for example, interleukin (IL)-1α, IL-6 or thymic stromal lymphopoietin (TSLP), considerably contribute to the pathogenesis (3–5).

An intact epidermal structure is a prerequisite for the skin to function adequately as a physical and chemical barrier. In AD, this skin barrier function is disturbed. Changes in skin ceramides and the altered expression of enzymes involved in the development of epidermal adhesion structures contribute to the breakdown of the epidermal barrier in AD patients (1). The impact of a mutation in FLG on the pathogenesis of AD has been highlighted — loss-of-function mutations in FLG are a strong predisposing factor for AD, and are the most-widely occuring genetic risk factor for AD known to date (6). Such mutations have been identified in up to 50% of all patients (7, 8). In addition, mutations in FLG not only predispose to AD, but can also cause ichthyosis vulgaris (9, 10). FLG loss-of-function mutations are known to provoke intercellular barrier abnormality, reducing the skin’s inflammatory threshold to topical irritants and antigens, which triggers inflammatory processes (10–12).

Despite the high prevalence of AD, valid data on the absorption of drugs/xenobiotics in diseased skin are lacking. Most likely, absorption in diseased skin differs from that of healthy skin due to the altered barrier function, possibly resulting in the higher systemic availability of, for example, topically applied drugs or environmental compounds. To improve the understanding of AD and the development of new treatment options, and for hazard analysis, non-clinical studies have to